Rejection of Claims 8, 26, 39, 42, 43, and 49 Under 35 U.S.C. §102(b) in view of Beutler *et al.* (U.S. Patent 5,447,851)

The Examiner has rejected claims 8, 26, 39, 42, 43, and 49 as being anticipated by Beutler *et al.* (U.S. Patent 5, 447,851; hereinafter Beutler) as evidenced by Invitrogen Life Technologies Manual (*Baculodirect™ Baculovirus Expression System,* 2004; Version F: 1-64; hereinafter Invitrogen manual). The Examiner states that while Beutler does not teach specific cell culturing temperatures, Beutler describes culturing of insect cells and, "therefore, the preparation taught by Beutler *et al.* would produce at least 70% biologically active fusion protein and no more than 30% inactive protein." Applicants respectfully traverse this rejection.

Claims 8 and 26 are directed to a high yield preparation enriched in biologically active receptor-Ig protein comprising at least 70% biologically active receptor-Ig fusion protein and no more than 30% inactive receptor-Ig fusion protein. Such improved preparations are obtained by culturing either a **mammalian or yeast cell** in a culture system having a specified temperature range. The recited temperature ranges are reduced relative to conventional temperatures known in the art for each particular cell type, *i.e,* about 27° C to about 35° C for mammalian cells, which have a conventional culturing temperature of 37° C (claim 8), and about 10° C to about 25° C for yeast cells, which have a conventional culturing temperature of 30° C (claim 26). Dependent claim 39 is directed to a preparation obtained from a mammalian culture system having a temperature of about 27° C to about 32° C. Dependent claim 42 is directed to a preparation which is a cell culture supernatant, while dependent claim 43 requires the preparation to comprise at least 83% biologically active receptor-Ig fusion protein. Claim 49 is directed to a highly enriched cell culture supernatant obtained by culturing a mammalian host cell, wherein the supernatant has improved ligand binding relative to a high temperature supernatant obtained by culturing a mammalian host cell transformed with DNA encoding the receptor-Ig fusion protein in a culture system having a temperature greater than about 35° C.

Beutler describes chimeric polypeptides consisting of immunoglobulin fusion proteins which may contain a cleavable linker peptide. As described by the Examiner, Beutler

suggests producing the chimeric polypeptides in either prokaryotic or eukaryotic cells, such as CHO and insect cells. Despite the fact that the Examiner acknowledges that Beutler does **not** "specifically teach the temperature at which the cells producing the fusion protein are cultured," the Examiner asserts that the claimed invention is anticipated by Beutler because insect cells are used and "the cells are incubated...at 27 °C." In support of Examiner's assertion that the insect cells are cultured at 27 °C, the Examiner cites an Invitrogen manual which details the **insect cell-based system** *Baculodirect™ Baculovirus Expression System.* The manual adds nothing to explain the teachings of Beutler with regard to the present claims. The manual provided by the Examiner merely teaches that the **conventional** culturing temperature for culturing **insect** cells is 27 °C. The claims, on the other hand, relate to improved preparations produced in mammalian or yeast cells cultured under a specific, unconventional temperature range.

As described in more detail below, Beutler alone does not teach each and every element of the claimed invention required under 102. Applicants submit that the Examiner has improperly relied on the Invitrogen manual in combination with Beutler under 35 USC §102. In accordance with MPEP 2131.01, only one reference should be used in making a rejection under 35 U.S.C. 102. It is noted that a 35 U.S.C. §102 rejection over multiple references may be proper when the extra reference is cited to: prove the primary reference contains an "enabled disclosure;" explain the meaning of a term used in the primary reference; or show that a characteristic not disclosed in the reference is inherent. The Examiner has not indicated where in Beutler each of the elements described in the pending claims can be found, instead stating that "[t]he claimed preparation **appears** to be the same as the prior art" (emphasis added). The Invitrogen manual cannot be used to show a characteristic is inherent in the teachings of Beutler, because Beutler alone is deficient in teaching each and every element (either explicitly or inherently) of the rejected claims, as is required under a 35 U.S.C. §102. Furthermore, the Invitrogen manual has not been referenced for any of the cited reasons as being necessary to combine with Beutler making the combination of the two references improper under 102. Under 35 U.S.C. §102, for a prior art reference to anticipate a claimed invention, the prior art must teach ***each and every***

element of the claimed invention. *Lewmar Marine v. Barient*, 827 F.2d 744, 3 USPQ2d 1766 (Fed. Cir. 1987). Furthermore, "the identical invention must be shown in as complete detail as is contained in the...claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989). Applicants respectfully submit that the Examiner has failed to establish how Beutler teaches each and every element of the claimed invention in accordance with 35 U.S.C. §102.

Applicants respectfully point out that the Examiner has overlooked significant features of the invention which are required elements of the pending claims and are not taught by Beutler. Applicants' discovery is based on benefits, *i.e.*, improved preparations of receptor-Ig-fusion protein having an increased percentage of biologically active protein, achieved by lowering the culture temperature of a transformed host cell relative to the accepted standard protocol temperature. The claimed improved preparations are possible because of this discovery. *The process by which the improved claimed preparations are made* ***(culturing below the conventional temperature) is a required limitation of the pending claims***. Beutler fails to teach this important feature of the claimed invention, as Beutler is silent as to culturing temperatures and further does not teach preparations having improved biological activity obtained by decreasing the culturing temperature.

Another significant feature which is a required element of the pending claims is that the claimed preparations and cell culture supernatants include a certain percentage of biologically active receptor-Ig proteins as a result of the culturing conditions described above. Claims 8, 26, 39, 42, and 49 each require that the preparations or cell culture supernatants include at least 70% biologically active receptor-Ig fusion protein and no more than 30% inactive receptor-Ig fusion protein. Claim 43 requires the preparation include at least 83% biologically active receptor-Ig fusion protein. Beutler does not teach a preparation or cell culture supernatant which includes either at least 70% or at least 83% biologically active receptor-Ig fusion proteins. Anticipation under 102 is based on a cited art reference teaching each and every element of the claimed invention, and not on assumptions by the Examiner as to what a reference appears to teach. Accordingly, should the Examiner

maintain this rejection, the Examiner is respectfully requested to provide specific reference to the teachings in Beutler which anticipate each and every element of the claimed invention.

In fact, the Examiner has acknowledged that Beutler is silent as to culture temperature conditions. Beutler does not incorporate the teachings of the Invitrogen manual into the patent, so it is guesswork by the Examiner as to the culture temperature conditions used by Beutler. In addition, the Examiner has failed to provide any evidence that the protocols of the Invitrogen manual were followed by Beutler, nor that the protocols in the manual, if followed, would result in an increased percentage of biologically active receptor-Ig fusion proteins, as required by the claimed invention. The manual provided by the Examiner merely shows that the standard protocol temperature for culturing insect cells is 27 °C. Finally, the Examiner has provided no evidence that insect cell cultures described in Beutler would be equivalent to the mammalian and yeast preparations of the claimed invention. Applicants request clarification as to whether the Examiner is taking the position that glycoproteins in insect cell preparations are the same as mammalian and yeast cells, and, if so, provide evidence supporting such an assertion.

In summary, the Examiner has failed to establish anticipation by Beutler because Beutler does not teach each and every element of the rejected claims. As such, it is improper for the Examiner to place the burden on Applicants to show that the claimed product is different from the art. Nonetheless, Applicants point out that the instant specification provides a controlled experiment which contrasts preparations obtained using conventional standards versus those made at temperatures lower than the conventional temperature. Table II at page 25 of the specification describes the percentage of inactive receptor-Ig fusion protein in cultures obtained from CHO cells. Table II illustrates that a preparation obtained by culturing the mammalian CHO cells at the standard culture temperature of 37 °C comprises 50% inactive LT-β-R-Ig protein. Table II shows in contrast that a preparation obtained by culturing mammalian CHO cells at a temperature ***below the standard culture temperature***, *i.e.*, 32 °C or 28 °C, comprises an improved 17% and 10% inactive LT-β-R-Ig protein, respectively. The Examiner has provided no evidence that mammalian cells referred to in Beutler would be cultured at anything other than the standard temperature. ***Applicants***

provide data in the instant specification which describes the activity differences between the standard temperature and the low temperature method preparations claimed herein.

In conclusion, to serve as an anticipation rejection, a reference must disclose each and every limitation of the claimed invention. Beutler fails to teach culturing either mammalian or yeast cells at a lower temperature relative to conventional temperatures in order to increase the percentage of biologically active receptor-Ig fusion proteins. Beutler is deficient in teaching each and every element (either explicitly or inherently) of the claimed invention; therefore, it is improper under 102 to rely on the Invitrogen manual since the primary reference fails to meet the standards set forth under 102. It is improper under 102 to rely on the Invitrogen manual to provide a missing element. Applicants respectfully submit that Beutler fails to anticipate the claimed invention, either explicitly or inherently, and request that this rejection be withdrawn.

Rejection of Claims 8, 11, 26, 29, 37, 38-40, 42-43, and 49 Under 35 U.S.C. §102(b) in view of Ashkenazi *et al.* (WO 98/25967)

The Examiner has rejected claims 8, 11, 26, 29, 37, 38-40, 42, 43, and 49 as being anticipated by Ashkenazi *et al.* (WO 98/25967; hereinafter Ashkenazi) as evidenced by Invitrogen Life Technologies Manual (*Baculodirect™ Baculovirus Expression System*, 2004; Version F: 1-64; hereinafter Invitrogen manual). The Examiner states that Ashkenazi does not "specifically teach the temperature at which the cells producing the fusion protein are cultured," but that Ashkenazi describes CHO and baculovirus expression systems. The Examiner suggests that the preparation taught by Ashkenazi "would produce at least 70% biologically active fusion protein and no more than 30% inactive protein" because insect cells are cultured at 27 °C, as evidenced by the Invitrogen manual. Applicants traverse this rejection.

To serve as an anticipation rejection under 102, a reference must disclose *each and every* limitation of the claimed invention. Further, to serve as an anticipation reference in an inherency rejection, the reference must make clear that the missing descriptive matter is necessarily present in the thing described in the reference. *Schering Corporation v. Geneva*

Pharmaceuticals, Inc., 339 F.3d 1373, 1376 (Fed. Cir. 2003). Applicants submit that Askenazi fails to teach each and every element of the claimed invention, and, furthermore, that it is improper for the Examiner to rely on the Invitrogen manual to provide a missing element from Ashkenazi.

As described above, the pending claims are directed to preparations and cell culture supernatants which are obtained from mammalian or yeast cells cultured at temperatures which are below the convention culturing temperature. Applicants' invention is based on the discovery that reducing the culturing temperature relative to the conventional standard temperature results in an increased percentage of biologically active receptor-Ig fusion protein. As such, the pending claims require that the preparations or supernatants comprise at least 70% biologically active receptor-Ig fusion protein.

Ashkenazi describes HVEM polypeptides, including nucleic acids encoding said and chimeric proteins. Applicants do not agree with the Examiner's reasoning that protein preparations described in Ashkenazi anticipate the claimed invention for the same reasons presented above in reference to the Beutler reference, the substance of which is reiterated here. Askenazi does not teach essential elements of the claimed invention as required under 102. Applicants' discovery is based on benefits, *i.e.*, preparations with increased percentages of biologically active receptor-Ig-fusion protein, achieved by lowering the culture temperature of a transformed host cell relative to the accepted standard protocol temperature. ***Applicants' discovery of the benefits of culturing below the conventional temperature is a required limitation of the pending claims***. As such, the claims are limited to preparations and supernatants from mammalian cells obtained from cultures grown at a temperature below the conventional 37° C, *i.e.*, about 27° C to about 35° C. The claims also require that preparations and cultures obtained from yeast cells be grown at a temperature below the conventional 30° C, *i.e.*, about 10° C to about 25° C. Furthermore, also reflective of Applicants' discovery of the benefits of low temperature culture conditions, the pending claims require that the preparations comprise at least 70% biologically active receptor-Ig fusion protein. Ashkenazi does teach improved cultures obtained from culturing mammalian or yeast cells at a low temperature. Nor does Askenazi teach preparations or supernatants

comprising at least 70% biologically active receptor-Ig fusion protein. Thus, Ashkenazi fails to teach each and every element of the claimed invention under 102 and does not anticipate the claimed invention.

As described by the Examiner, Ashkenazi does "not specifically teach the temperature at which the cells...are cultured." As described above, it is improper to rely on the Invitrogen manual to provide a missing element to Ashkenazi. While the missing element can be inherent and another reference can be relied on to show that the element was inherent, in this instance Ashkenazi is deficient in teaching each and every element either explicitly or inherently. Therefore, the 102 rejection based on the primary reference is improper and the Invitrogen manual cannot be relied on to provide the missing element.

Applicants respectfully submit that the Examiner has failed to establish that the compositions described in Ashkenazi would have at least 70% biologically active receptor-Ig fusion protein as required by the pending claims, or that they would be obtained from a mammalian or yeast cell culture which would result in such a high percentage of active protein. In view of the foregoing, Applicants respectfully submit that Ashkenazi fails to anticipate the claimed invention, either explicitly or inherently and request that this rejection be withdrawn.

Rejection of Claims 8, 10, 11, 16, 26, 28-29, and 37-51 Under 35 U.S.C. §103(a) over Crowe *et al.*, Rennert *et al.*, and Kwon *et al.* in view of Nilsson *et al.* or Beutler *et al.* (U.S. Patent 5,447,851)

The Examiner has rejected claims 8, 10, 11, 16, 26, 28-29, and 37-51 as being anticipated by Crowe *et al.* (1994) *Science* 264:707 (hereinafter Crowe-Science); Kwon *et al.* (1997) *J. Biol. Chem.* 272:14272 (hereinafter Kwon); or Rennert *et al.* (1996) *J. Exp. Med.* 184:1999 (hereinafter Rennert) in view of Nilsson *et al.* (*Protein Expr. Purif.* (1997) 11:16; hereinafter Nilsson) or Beutler *et al.* (US Patent 5,447,851; hereinafter Beutler). The Examiner states that Crowe-Science, Kwon, and Rennert "do not specifically teach a high yield preparation comprising at least 70% biologically active receptor-Ig fusion protein." In view of this deficiency, the Examiner cites Beutler and Nilsson as providing motivation. The Examiner suggests that "[i]t would have been *prima facie* obvious to one of ordinary skill in

the art at the time the invention was made to make a high yield preparation comprising an Ig-receptor fusion protein that consists of at least 70% active fusion protein." Applicants respectfully traverse this rejection.

A proper *prima facie* obviousness rejection requires that there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Additionally, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. See M.P.E.P. § 2143. Also, see In re Vaeck, 947 F.2d 488, 493, 20 U.S.P.Q.2d 1438, 1443 (Fed. Cir. 1991) (the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure).

Applicants submit that the cited references when combined do not teach or suggest all the claim limitations, as required under 35 USC §103. As acknowledged by the Examiner, Crowe-Science, Kwon, and Rennert "do not specifically teach a high yield preparation comprising at least 70% biologically active receptor-Ig fusion protein" as required by the pending claims. The references cited by the Examiner to account for this deficiency do not teach this required element of Applicants' invention either.

Nilsson teaches fusion proteins which are used as affinity tags, referred to as "affinity fusion technology" by the authors of the Nilsson reference. The Nilsson reference provides an overall review of fusion proteins, especially proteins containing an affinity tag, for use as purification reagents in protein columns and as detection reagents. Nilsson provides a survey of the more commonly used affinity tag proteins, including polyhistidine tags, biotinylated tags, GST tags, and FLAG peptides. Nilsson further provides suggestions for cleaving fusion proteins after purification. Nilsson summarizes the review at page 11, stating "This review points out new strategies based on affinity fusions, such as the use of affinity-tagged proteases and affinity assisted *in vivo* folding, which would facilitate down-stream bioprocessing by circumventing some of the problems relating to proteolytic cleavage of fusion proteins, and this decrease overall production costs." Beutler, as described above,

merely refers to certain host cells which are preferential for protein expression and does not teach or suggest modifications of such culture systems. None of the cited references either alone or in combination teach a preparation obtained from a culture grown at a temperature lower than conventionally used with at least 70% biologically active receptor-Ig fusion protein. Thus, neither Beutler nor Nilsson alone or in combination with Crowe-Science, Kwon, or Rennert teaches all of the required elements of the claims.

In contrast to the Examiner's assertion, neither Beutler nor Nilsson provide motivation to produce a preparation comprising a high percentage of biologically active receptor-Ig protein. Beutler merely teaches the benefits of culturing eukaryotic cells over prokaryotic cells, stating at col. 9, lines 60-65,

> The method of producing the chimeric polypeptides of the Invention may utilize a eukaryotic cell such as a CHO or insect cell or it may utilize a prokaryotic [sic] cell. However, in order to maximize the amount of active polypeptide recovered from such cells, eukaryotic cells are preferred.

Beutler teaches that the selection of the host cell should be dictated by cells that produce the most active polypeptide. The teaching in Beutler would not motivate one of ordinary skill in the art to modify the culturing conditions of a given system, but rather would draw one's attention to the inherent differences among cell-based expression systems, such as the differences between mammalian and insect cells. The Nilsson reference does not teach or suggest culturing methods or modifications thereof, including those relating to temperature shifting as claimed in the instant invention, which would lead one of ordinary skill in the art to arrive at the claimed invention. Thus, none of the cited references, Kwon, Crowe-Science, or Rennert in view of either Nilsson or Beutler provides any motivation to one of ordinary skill in the art as none of the references teach or suggest modifying culturing conditions to arrive at a preparation which has an increased level of biologically active protein.

The last element to establishing a *prima* facie case of obviousness is that there must be a reasonable expectation of success. The claimed invention is directed to high yield preparations and pharmaceutical compositions obtained by culturing a host cell at a

temperature lower than the standard temperature in order to increase the percentage of biologically active receptor-Ig fusion proteins. The references and comments provided by the Examiner relate to systems and methods of purifying proteins which are not relevant to the claimed invention. Based on the references provided by the Examiner, one of ordinary skill in the art would be led to believe that a preparation must be purified in order to increase the percentage of active molecules. Importantly, Applicants' invention provides preparations comprising increased percentages of biologically active fusion proteins as the protein is expressed and not due to a post-culture procedure or final purification. None of the references cited by the Examiner alone or in combination provide an expectation of success to one of ordinary skill that modifications to the culture conditions can improve the percentage of biologically active fusion proteins.

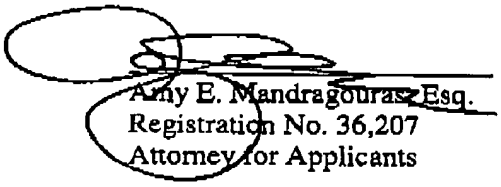
In view of the foregoing, Applicants submit that neither Nilsson nor Beutler cures the deficiencies of Crowe-Science, Kwon, and Rennert. Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

In view of the foregoing comments, reconsideration of the rejections and allowance of all pending claims is respectfully requested.

If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' Attorney at (617) 227-7400.

Respectfully submitted,



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Dated: November 22, 2005